

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of )  
)  
Richard DEROSE et al. ) Group Art Unit: 1638  
)  
Continuation of Application )  
Serial No. 09/000,062, ) Examiner: O. Zaghmout  
filed May 29, 1998 )  
)  
Filed: December 21, 2001 )  
)  
For: ISOLATED DNA SEQUENCE )  
CAPABLE OF SERVING AS )  
REGULATORY ELEMENT IN A )  
CHIMERIC GENE WHICH CAN )  
BE USED FOR THE )  
TRANSFORMATION OF PLANTS)

**PRELIMINARY AMENDMENT**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Prior to examination of the subject application on the merits, please amend the above-identified application as follows:

**IN THE SPECIFICATION:**

Please replace the paragraph beginning at page 11, line 8, with the following:

-- EXAMPLE 1:

1. Production of an EPSPS fragment from Arabidopsis thaliana

a) two 20-mer oligonucleotides of respective sequences (SEQ ID NOS: 8 and 9):

5'-GCTCTGCTCATGTCTGCTCC-3'

5'-GCCCGCCCTTGACAAAGAAA-3'

were synthesized from the sequence of an EPSPS gene from Arabidopsis thaliana (Klee H.J. et al., (1987) Mol. Gen. Genet., 210, 437-442). These two oligonucleotides correspond to positions 1523 to 1543 and 1737 to 1717, respectively, of the published sequence and in convergent orientation.--

Please replace the paragraph beginning at page 13, line 3, with the following:

--Two single-stranded and partially complementary oligonucleotides of respective sequences (SEQ ID NO:10):

5'-AATTCCCGGG-3'

5'-CCCGGG-3' (the latter being phosphorylated)

are ligated to double-stranded cDNAs with blunt ends.--

Please replace the paragraph beginning at page 20, line 11, with the following:

--The plasmid DNA content of the various clones was analysed according to the procedure described for pRPA-ML-711. One of the plasmid clones retained contains an EcoRI-HindIII insert of about 1.45 kbp. The sequence of the terminal ends of this clone shows that the 5' end of the insert corresponds exactly to the corresponding end of pRPA-ML-711 and that the 3' terminal end has the following sequence (SEQ ID NO:11):

"5'...AATTAAGCTCTAGAGTCGACCTGCAGGCATGCAAGCTT-3'".--

Please replace the paragraph beginning at page 21, line 3, with the following:

--The clone pRPA-ML-712 was cut with the restriction enzymes PstI and HindIII. The DNA resulting from these manipulations was separated by electrophoresis on a 0.8 % LGTA/TBE agarose gel (ref. CPMB). The gel fragment containing the PstI/EcoRI insert of 1.3 kbp was excised from the gel and purified according to the procedure described in paragraph 5 above. This insert was ligated in the presence of an equimolar quantity of each of the two partially complementary oligonucleotides of sequence (SEQ ID NOS:12 and 13):

Oligo 1: 5'-GAGCCGAGCTCCATGGCCGGCGCCGAGGAGATCGTGCTGCA-3'

Oligo 2: 5'-GCACGATCTCCTCGGCGCCGGCCATGGAGCTCGGCTC-3'

as well as in the presence of DNA from the plasmid pUC19 digested with the restriction enzymes BamHI and HindIII.--

Please replace the paragraph beginning at page 24, line 16, with the following:

--The sequence of pRPA-ML-715 is arbitrarily numbered by placing the first base of the N-terminal alanine codon GCC in position 1. This sequence has an NcoI site in position 1217. The site-modifying oligonucleotide has the sequence (SEQ ID NO:14):

5'-CCACAGGATGGCGATGGCCTTCTCC-3'.--

Please replace the paragraph beginning at page 25, line 7, with the following:

--The following oligonucleotides (SEQ ID NOS:15, 16, 17 and 18) were used:

a) Thr 102 → Ile mutation.

5'-GAATGCTGGAATCGCAATGCGGCCATTGACAGC-3'

b) Pro 106 → Ser mutation.

5'-GAATGCTGGAAGTCAATGCGGTCCTTGACAGC-3'

c) Gly 101 → Ala and Thr 102 → Ile mutations.

5'-CTTGGGGAATGCTGCCATCGCAATGCGGCCATTG-3'

d) Thr 102 → Ile and Pro 106 → Ser mutations.

5'GGGGAATGCTGGAATCGCAATGCGGTCCTTGACAGC-3'--

Please replace the paragraph beginning at page 26, line 17, with the following:

--A DNA fragment of 418 base pairs is purified from digestion of the cosmid clone c22 with the restriction enzyme DdeI followed by treatment with a Klenow fragment of DNA polymerase from E. coli, according to the manufacturer's instructions for creating a blunt-ended DNA fragment and then cut with MseI. The purified DNA fragment is ligated to a synthetic oligonucleotide adaptor having the following sequence (SEQ ID NOS:19 and 20):

Adaptor 1: 5' TAATTTGTTGAACAGATCCC 3'

TAAACAACCTTGCTAGGG--

Please replace the paragraph beginning at page 27, line 5, with the following:

--3. Intron No. 2:

A DNA fragment of 494 base pairs is purified from the digestion of the cosmid clone c22 with the restriction enzymes AluI and CfoI. The purified DNA fragment is ligated to a synthetic oligonucleotide adaptor having the following sequence (SEQ ID NOS:21 and 22):

Adaptor 2: 5' CAGATCCCGGGATCTGCG 3'

GCGTCTAGGGCCCTAGACGC--

In compliance with 37 C.F.R. § 1.823(a), please substitute the attached paper copy of the Sequence Listing for the originally filed Sequence Listing on pages 37-45 of the specification. Kindly renumber the pages of the specification accordingly.

**IN THE CLAIMS:**

Please cancel claims 2-24 without prejudice or disclaimer of the subject matter recited therein.

Please replace claim 1 with the following amended claim:

1. (Amended) An isolated DNA sequence serving as a genetic regulatory element in a chimeric gene, wherein said DNA sequence is the first intron (intron 1) of the 5' non-translated region of a plant H3.3 histone gene.

**IN THE ABSTRACT:**

Please delete the Abstract and insert the enclosed Abstract, which is properly on a separate sheet of paper, in place thereof.

**REMARKS**

Entry of the foregoing, examination and favorable consideration of the subject application in light of the following remarks are respectfully requested.

By the foregoing amendment, the specification has been amended to insert the appropriate sequence identifiers. Further, the specification has been amended to insert the attached copy of the sequence listing after the last page of the disclosure (currently page 36) and before the claims (currently page 46). Please renumber the pages accordingly. Claims 2-24 have been canceled without prejudice or disclaimer of the subject matter recited therein. Further, claim 1 has been amended to further clarify Applicant's invention. Support for the amendments can be found throughout the specification. Accordingly, no new matter has been added.

In view of the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order. Such action is earnestly solicited.

In the event that there are any questions relating to this application, it would be appreciated if the Examiner would telephone the undersigned attorney concerning such questions so that prosecution of this application may be expedited.

Respectfully submitted,

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Date: December 21, 2001

**Attachment to Amendment dated December 21, 2001**  
**Marked-up Claim**

1. (Amended) An isolated DNA sequence [capable of] serving as a genetic regulatory element in a chimeric gene, wherein said DNA sequence is the first intron (intron 1) of the 5' non-translated region of a plant H3.3 histone gene [which can be used for the transformation of plants and allowing the expression of the product of translation of the chimeric gene in particular in the regions of the plant undergoing rapid growth, characterized in that it comprises at least one intron such as the first intron (intron 1) of the noncoding 5' region of a plant histone gene].



**Attachment to Amendment dated December 21, 2001**

**Marked-up Specification**

**Paragraph beginning at page 11, line 8:**

**EXAMPLE 1:**

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c) Gly 101 → Ala and Thr 102 → Ile mutations.

5'-CTTGGGGAATGCTGCCATCGCAATGCGGCCATTG-3'

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Adaptor 1: 5' TAATTTGTTGAACAGATCCC 3'

TAAACAACCTGTCTAGGG

**Paragraph beginning at page 27, line 5:**

3. Intron No. 2:

A DNA fragment of 494 base pairs is purified from the digestion of the cosmid clone c22 with the restriction enzymes AluI and CfoI. The purified DNA fragment is ligated to a synthetic oligonucleotide adaptor having the following sequence (SEQ ID NOS:21 and 22):

Adaptor 2: 5' CAGATCCCGGGATCTGCG 3'

GCGTCTAGGGCCCTAGACGC

## ABSTRACT

An isolated DNA sequence capable of serving as regulatory element in a chimeric gene which can be used for the transformation of plants is disclosed. A chimeric gene for the transformation of plants is also disclosed. The gene comprises at least, in the direction of transcription, a promoter sequence, a transgene and a regulatory element, characterized in that the regulatory element consists of at least one intron 1 in the noncoding 5' region of a plant histone gene allowing the expression of the protein in the zones undergoing rapid growth. The production of transgenic plants is also disclosed.